

A Comparison of the Immune Response of 2003 Commercial Turkeys and a 1966 Randombred Strain When Fed Representative 2003 and 1966 Turkey Diets¹

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ABSTRACT The immunological performance of modern turkeys (one-third each of the Nicholas Turkey, British United Turkeys of America, and Hybrid Turkey strains) hatched in 2003 (2003 strain) was compared with that of a randombred control turkey strain (RBC2) established in calendar year 1966, when fed representative 1966 and 2003 type diets. The 2003 strain had a higher BW and bursa of Fabricius weight relative to total BW compared with the RBC2 strain ($P = 0.0001$) when measured at 12 and 13 d of age, respectively. Total antibody response against SRBC did not differ between strains, nor were any differences observed in the IgM antibody levels either during a primary or secondary SRBC challenge. However, RBC2 poults had higher IgG levels ($P = 0.02$) than the 2003 strain at 7 d post secondary SRBC challenge. No

significant differences were observed in the phytohemagglutinin phosphate-mediated toe-web lymphoblastic response. However, the 2003-strain turkeys seemed to have a better swelling response ($P = 0.06$) than the RBC2-strain turkeys when measured at 24 h post phytohemagglutinin phosphate injection. The modern turkeys also had higher mononuclear phagocytic system function, as measured by clearance of carbon particles from the bloodstream 5 min post intravenous injection of colloidal carbon ($P = 0.02$). These results indicate that selection over the years of turkeys for improved performance traits has had no adverse effects on most of the immune system indicators when examined prior to sexual maturity in the current study.

Key words: genetic change, immunological change, diet, turkey

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INTRODUCTION

Selection of turkey lines for increased growth and reproductive performance has been shown to negatively affect the immune functions of birds. For example, a turkey line (line E) selected for high egg production had a higher mortality rate from fowl cholera and poorer antibody responses to Newcastle disease virus (NDV) and *Pasteurella multocida* in comparison with its progenitor randombred control line (Saif et al., 1984; Sharaf et al., 1998). Nestor et al. (1996) found similar results in a turkey line selected for increased growth rate at 16 wk of age (i.e., the F line) which exhibited decreased resistance to *P. multocida* and NDV in comparison with the control line from which it was derived (i.e., RBC2). Mortality to NDV was also sig-

nificantly higher in the F line in comparison with line E, which had been selected long-term for increased egg production, and its randombred control strain (i.e., RBC1; Tsai et al., 1992). Bayyari et al. (1997) showed that the cell-mediated immune response, as detected by using the toe-web response to phytohemagglutinin phosphate (PHA-P) injection, lymphocyte count, and relative spleen weight, was poorer in the F line than in the RBC2 line. These results suggest that selection for a faster growth rate is accompanied by changes in the humoral and cell-mediated immune response that may potentially affect the overall immune response. The objective of the present study was to compare the immune competence of modern commercial turkeys from the 2003 calendar year (one-third each of the Nicholas Turkey, British United Turkeys of America, and Hybrid Turkey strains) and a randombred control line (RBC2) that was established in calendar year 1966, when the birds were raised on diets representative of those used by the industry in 2003 and 1966. A number of immunological assays were used, including the humoral response against SRBC, lymphoproliferative response to PHA-P, and mononuclear phagocytic system function of clearing colloidal carbon from the blood circulation, an indication

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Table 1. Body weights and percentage of relative bursa weights¹ of modern commercial 2003 turkeys and 1966 randombred turkeys when fed representative 1966 and 2003 diets by strain, age, and diet

Item	Diet ³	Sex	BW (g)	Bursa weight (%)
Strain ²				
2003	2003	Males	390.6	0.66
2003	1966	Males	266.8	0.55
1966	2003	Males	210.8	0.34
1966	1966	Males	288.2	0.45
2003	2003	Females	380.3	0.64
2003	1966	Females	251.5	0.60
1966	2003	Females	169.7	0.36
1966	1966	Females	237.3	0.40
Strain average				
2003			322.3	0.61
1966			226.5	0.39
Pooled SEM			13.5	0.06
Source of variation				
Strain			0.0001	0.0001
Diet			0.01	0.9
Sex			0.005	0.9
Strain × diet			0.0001	0.07
Strain × sex			0.11	0.73
Sex × diet			0.71	0.97
Strain × sex × diet			0.91	0.45

¹ Average BW and bursa weights as a percentage of BW from 6 poult groups taken at 12 and 13 d of age.

² 2003 = composite of 2 poult groups each of Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains; 1966 = randombred control strain (RBC2) from The Ohio State University (Wooster, OH).

³ 2003 = turkey dietary regimen representative of those being fed in the 2003 calendar year; 1966 = turkey dietary regimen representative of those being fed in 1966, adapted from Ensinger (1967). Both starter diets were fed as crumbles.

of phagocytic potential. Body weights and bursal weights relative to total BW were also measured.

MATERIALS AND METHODS

Strains and Strain Management

Newly hatched and sexed poult groups were obtained from the 3 primary commercial strains (i.e., Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains), and sexed poult groups were obtained from the RBC2 line of turkeys (kindly provided by Karl E. Nestor, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH). As described in detail in Havenstein et al. (2006), all poult groups in the primary growth study were neck tagged and placed in 32 pens using a 2 × 2 × 2 factorial arrangement [i.e., 2 strains (modern 2003 and RBC2), 2 sexes, and 2 dietary regimens (2003 and 1966 turkey diets)] in a single house at the Turkey Educational Unit, North Carolina State University (Raleigh, NC). Details of the management practices and dietary regimens used are fully described in Havenstein et al. (2007).

Because the poult groups for the mononuclear phagocytic system function assay had to be killed, they were individually identified at hatch and were placed with the sexes together on the appropriate dietary treatments in 4 pens that were in the same facility as, but which were separate from, the 32 pens used in the main study described by Havenstein et al. (2007). Each pen had a total of 16 RBC2 and 48

modern-strain poult groups per pen. The antibody response and lymphoproliferative response assays were performed on 8 poult groups per group from the main 32-pen study (Havenstein et al., 2007). The 1966 and 2003 diets involved are fully described in Havenstein et al. (2007), and the first four 2003 diets that were used through 7 wk of age for the current part of the study had an average of 3,000 kcal of ME/kg and 23.75% CP. The 1966 starter diet that was used for this part of the study had 2,800 kcal of ME/kg and 29% CP for the same time period.

BW and Percentage Relative Bursa Weights

The BW and bursa of Fabricius weights were measured from 6 poult groups per group at 12 and 13 d of age. Bursa weights were expressed as a percentage of live BW.

Antibody Response

Two-week-old poult groups were injected intravenously via the jugular vein with a 1-mL volume of a 5% SRBC suspension (d 0, primary injection), followed by a second injection (boost) at 4 wk of age (d 14). Blood samples from 8 poult groups per strain-sex-diet subgroup were collected at 7 and 14 d after the primary injection and again at 7 and 14 d post-boost. The serum collected from each clotted blood sample was collected, heat inactivated at 56°C for 30 min, and then analyzed for total antibodies, mercaptoethanol-sensitive (MES; Sigma Chemical Co., St. Louis, MO) IgM anti-SRBC antibodies, and mercaptoethanol-resistant (MER) IgG anti-SRBC antibodies as previously described (Delhanty and Solomon, 1966; Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 µL of serum was added to an equal amount of PBS (Fisher Scientific, Pittsburgh, PA) in the first column of a 96-well V-shaped bottom plate (Corning, Corning, NY), and the solution was incubated for 30 min at 37°C. A serial dilution was then made (1:2) and 50 µL of a 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the end-point titer for agglutination. For the MES (IgM) response, 50 µL of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the aforementioned procedure. The difference between the total and the IgG response was considered to be equal to the IgM antibody level.

Lymphoproliferative Response to PHA-P

The lymphoproliferative response to PHA-P (Sanford, Bellwood, IL) was measured as an indicator of a T-cell-induced delayed-type hypersensitivity reaction, as described by Corrier (1990). The PHA-P was injected intradermally (100 µg/100 µL per poult) into the toe web of the left foot of 8 poult groups/group at 3 wk of age. The thickness of the toe webs was measured at 0 h of pre-PHA-P injection and then at 24 and 48 h using a micrometer. The swelling response was calculated as the percentage increase in toe-web thickness from the preinjection thickness.

Table 2. Total anti-SRBC antibody response¹ of modern commercial 2003 turkeys and 1966 randombred turkeys when fed representative 1966 and 2003 diets by strain, age, and diet

Item	Diet ²	Sex	Days PPI ³		Days PSI ⁴	
			7	14	7	14
Strain ⁵						
2003	2003	Males	6.65	2.00	5.26	2.19
2003	1966	Males	6.94	2.25	6.45	3.06
1966	2003	Males	6.87	2.25	5.62	3.00
1966	1966	Males	6.75	2.50	6.37	3.12
2003	2003	Females	7.19	2.12	4.59	2.18
2003	1966	Females	7.38	2.44	5.88	3.06
1966	2003	Females	6.62	1.75	4.87	1.88
1966	1966	Females	7.37	3.12	5.62	2.50
Strain average						
2003			7.04	2.20	5.54	2.62
1966			6.90	2.40	5.62	2.62
Pooled SEM			0.39	0.31	0.46	0.26
Source of variation						
Strain			0.64	0.37	0.76	0.99
Diet			0.34	0.02	0.0004	0.001
Sex			0.24	0.63	0.01	0.02
Strain × diet			0.89	0.25	0.37	0.19
Strain × sex			0.60	0.84	0.81	0.02
Sex × diet			0.49	0.19	0.93	0.51
Strain × sex × diet			0.39	0.25	0.93	0.53

¹Two-week-old poults were given a 5% SRBC injection (d 0) followed by a second injection at 4 wk of age (d 14). Serum samples from 8 poults per strain-diet-sex subgroup were collected at 7 and 14 d after the primary injection and again at 7 and 14 d postboost. Serum samples were analyzed for the presence of total anti-SRBC antibodies. The data represent the mean \pm SE of log₂ of the reciprocal of the last dilution exhibiting agglutination.

²2003 = turkey dietary regimen representative of those being fed in the 2003 calendar year; 1966 = turkey dietary regimen representative of those being fed in 1966, adapted from Ensinger (1967). Both starter diets were fed as crumbles.

³Days PPI = days post primary injection.

⁴Days PSI = days post secondary injection.

⁵2003 = composite of 2 poults each of Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains; 1966 = randombred control strain (RBC2) from The Ohio Agricultural Research and Development Center (Wooster, OH).

Mononuclear Phagocytic System Function Assessment

Colloidal carbon (black india ink; Sanford, Bellwood, IL) was injected into the brachial vein of 6 poults/group at 100 μ L/bird at 12 and 13 d of age. The concentration of carbon particles at 5- and 20-min intervals was measured in plasma samples via a microplate reader, as described by Cheng and Lamont (1988). The percentage increase in optical density (OD) of each sample, as an indicator of carbon levels over the baseline preinjection levels (i.e., corresponding plasma samples collected prior to the colloidal carbon injection), was calculated as follows: % OD = [(OD reading at a given time – OD reading at preinjection)/OD reading at preinjection] \times 100.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Institute, 1996). Strain, diet, sex, and the 2-way and 3-way interactions were included in all analyses. Means were separated for significance by Duncan's multiple-range test and were considered significantly different if the *P*-value was equal to or less than 0.05.

RESULTS AND DISCUSSION

BW and Relative Bursa Weights

The BW and percentage relative bursa of Fabricius weights were measured at 12 and 13 d of age and are presented in Table 1. The modern 2003 turkeys had significantly heavier BW than the RBC2 strain (*P* = 0.0001). Sex effects were observed for BW, with the males having higher BW than the females (*P* = 0.005). Turkeys reared for the first 14 d on the 2003 diet had significantly higher BW than those raised on the 1966 diets (*P* = 0.01). A significant strain \times diet effect was also observed, in which the birds from the 2 strains raised on the 2003 diets had a significantly larger difference in BW between them than did those reared on the 1966 diets (*P* = 0.0001).

The modern turkeys had significantly higher bursa of Fabricius weights relative to their BW than did the RBC2 strain (*P* = 0.0001, Table 1). The interaction between strain and diet approached significance (*P* = 0.07), indicating that the difference between the modern strain on the 2 diets (0.30%) was slightly larger than the difference between the RBC2 (0.26%) on the 2 diets.

The findings of this portion of the study are supported by the results of the main growth rate study (Havenstein

Table 3. Immunoglobulin M anti-SRBC antibody response¹ of modern commercial 2003 turkeys and 1966 randombred turkeys when fed representative 1966 and 2003 diets by strain, age, and diet

Item	Diet ²	Sex	Days PPI ³		Days PSI ⁴	
			7	14	7	14
Strain ⁵						
2003	2003	Males	6.31	1.97	4.48	2.06
2003	1966	Males	6.58	2.25	5.39	3.00
1966	2003	Males	6.88	2.25	4.25	2.62
1966	1966	Males	6.25	2.50	4.75	3.12
2003	2003	Females	5.75	2.06	3.88	2.06
2003	1966	Females	7.06	2.44	5.00	2.94
1966	2003	Females	6.62	1.62	3.62	1.38
1966	1966	Females	6.88	3.12	4.50	2.50
Strain average						
2003			6.42	2.18	4.69	2.52
1966			6.66	2.37	4.28	2.41
Pooled SEM			0.50	0.32	0.40	0.28
Source of variation						
Strain			0.53	0.39	0.17	0.59
Diet			0.41	0.01	0.004	0.0001
Sex			0.84	0.76	0.12	0.019
Strain × diet			0.18	0.24	0.58	0.82
Strain × sex			0.76	0.76	0.92	0.029
Sex × diet			0.19	0.15	0.61	0.48
Strain × sex × diet			0.91	0.21	0.89	0.41

¹Two-week-old poults were given a 5% SRBC injection (d 0) followed by a second injection at 4 wk of age (d 14). Serum samples from 8 birds per strain-diet-sex subgroup were collected at 7 and 14 d after the primary injection and again at 7 and 14 d postboost. Serum samples were analyzed for the presence of total anti-SRBC antibodies. The data represent mean \pm SE of log₂ of the reciprocal of the last dilution exhibiting agglutination.

²2003 = turkey dietary regimen representative of those being fed in the 2003 calendar year; 1966 = turkey dietary regimen representative of those being fed in 1966, adapted from Enslinger (1967). Both starter diets were fed as crumbles.

³Days PPI = days post primary injection.

⁴Days PSI = days post secondary injection.

⁵2003 = composite of 2 birds each of Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains; 1966 = randombred control strain (RBC2) from The Ohio Agricultural Research and Development Center (Wooster, OH).

et al., 2007), which showed that the growth rate to market age has approximately doubled for the modern commercial turkey over the 37-yr period from 1966 to 2003. The results from that part of the current study showed that the genetic selection practiced by the turkey breeding industry has resulted in the BW of commercial tom and hen turkeys at market ages having increased by approximately 208 and 140 g/yr, respectively, during this 37-yr period. It is interesting to note that the growth and development of the bursa of Fabricius was not negatively affected with the genetic selection practices related to improved BW (Table 1). The bursa of Fabricius is a key lymphoid organ that is responsible for the development and maturation of B-lymphocytes, and the humoral antibody response is dependent on this central organ (Zhang et al., 2006). For example, a high antibody response to SRBC has been associated with a larger bursa size in White Leghorn chicken strains (Ubosi et al., 1985). Furthermore, Zhang et al. (2006) showed a clear association between non-MHC genes and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines.

Antibody Response

The antibody responses were measured after the poults were injected with a 5% SRBC suspension at 2 wk of age

(d 0) and then boosted at 4 wk of age (d 14). Total, IgM, and IgG antibody responses (presented in Tables 2, 3, and 4, respectively) of the turkeys were measured from serum samples collected at 7 and 14 d post primary injection (PPI), and at 7 and 14 d post secondary injection (PSI). No significant strain differences were seen for the total anti-SRBC antibody response at any of the time points. Poults in all groups responded well to the first SRBC injection by mounting a peak antibody response (strain average range = 6.9 to 7.04 log₂; Table 2), which declined appreciably at 14 d PPI (strain average range = 2.2 to 2.4 log₂; Table 2). Upon booster SRBC injection, the antibody titers went up as expected at the peak time point (7 d PSI) and declined thereafter at 14 d PSI. Although the peak and decline of antibody titers during the induction and booster phases of antibody response are normal, why the antibody titers were not greater at 7 d PSI than the ones observed at 7 d PPI (Table 2) is difficult to explain. It must be pointed out that the source of SRBC as well as the poult husbandry conditions remained the same throughout the study. The toms had a significantly higher antibody response against SRBC than did the females at 7 and 14 d PSI ($P \leq 0.02$). A significant ($P = 0.03$) strain \times sex interaction for the antibody response was also present at 14 d PSI. This appears to have been caused by an unexplainably low measurement for the toms on the 1966 diet. Diet effects were ob-

Table 4. IgG anti-SRBC antibody response¹ of modern commercial 2003 turkeys and 1966 randombred turkeys when fed representative 1966 and 2003 diets by strain, age, and diet

Item	Diet ²	Sex	Days PPI ³		Days PSI ⁴	
			7	14	7	14
Strain ⁵						
2003	2003	Males	0.34	0.03	0.77	0.13
2003	1966	Males	0.35	0.00	1.06	0.06
1966	2003	Males	0.00	0.00	1.38	0.38
1966	1966	Males	0.50	0.00	1.62	0.00
2003	2003	Females	1.43	0.06	0.70	0.12
2003	1966	Females	0.31	0.00	0.88	0.12
1966	2003	Females	0.00	0.12	1.25	0.50
1966	1966	Females	0.50	0.00	1.12	0.00
Strain average						
2003			0.61	0.02	0.85	0.11
1966			0.25	0.03	1.34	0.22
Pooled SEM			0.34	0.04	0.27	0.09
Source of variation						
Strain			0.14	0.81	0.017	0.13
Diet			0.91	0.08	0.47	0.001
Sex			0.29	0.22	0.27	0.55
Strain × diet			0.03	0.81	0.68	0.005
Strain × sex			0.29	0.46	0.65	0.79
Sex × diet			0.25	0.22	0.54	0.85
Strain × sex × diet			0.25	0.46	0.75	0.50

¹Two-week-old poults were given a 5% SRBC injection (d 0) followed by a second injection at 4 wk of age (d 14). Serum samples from 8 poults per strain-diet-sex subgroup were collected at 7 and 14 d after the primary injection and again at 7 and 14 d postboost. Serum samples were analyzed for the presence of total anti-SRBC antibodies. The data represent mean ± SE of log₂ of the reciprocal of the last dilution exhibiting agglutination.

²2003 = turkey dietary regimen representative of those being fed in the 2003 calendar year; 1966 = turkey dietary regimen representative of those being fed in 1966, adapted from Ensminger (1967). Both starter diets were fed as crumbles.

³Days PPI = days post primary injection.

⁴Days PSI = days post secondary injection.

⁵2003 = composite of 2 poults each of Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains; 1966 = randombred control strain (RBC2) from The Ohio Agricultural Research and Development Center (Wooster, OH).

served at 14 d PPI and again at 7 and 14 d PSI, with the birds raised on the 1966 diets consistently showing greater antibody response than the birds raised on the 2003 diets ($P \leq 0.02$; Table 2).

No significant strain differences were observed for IgM antibody levels at any stage of the immunization regimen (Table 3). However, birds raised on the 1966 diets had significantly ($P \leq 0.01$) higher IgM titers against SRBC at 14 d PPI, and again at 7 and 14 d PSI than those fed the 2003 diets (Table 3). The males had significantly higher IgM titers than the females ($P = 0.02$) at 14 d PSI. The RBC2 males had significantly higher titers than the RBC2 females ($P = 0.03$) at 14 d PSI.

The RBC2 strain had significantly higher IgG titers than the modern strain at 7 d PSI ($P = 0.02$) but not at 7 and 14 d PPI or 14 d PSI (Table 4). The difference in IgG titers between birds raised on the 2003 diets compared with those raised on the 1966 diets approached significance ($P = 0.08$) at 14 d PPI, and was significantly ($P = 0.02$) higher at 14 d PSI. The modern birds on the 2003 diets had significantly ($P = 0.0340$) higher IgG titers than those reared on the 1966 diets at 7 d PPI. The RBC2 birds on the 2003 diets had significantly ($P = 0.005$) higher IgG titers than the RBC2 birds reared on the 1966 diet at 14 d PSI.

In previous studies, F line turkeys that had been selected for increased growth rate at 16 wk of age had higher total,

IgM, and IgG titers against SRBC than did the turkeys from the RBC2 line (Li et al., 2000a,b). At the same time, the F line turkeys had higher mortality and a shorter number of days to death following a challenge with *P. multocida* than did the RBC2 line. In another study (Cheema et al., 2003), a lower-BW 1957 Athens Canadian Randombred Control broiler strain had significantly higher total, IgM, and IgG responses to SRBC than did a heavy-BW 2001 Ross broiler strain. Results of the present study also indicate that selection for increased growth rate in commercial turkeys has decreased the IgG antibody response against SRBC. These observations suggest that although genetic selection for growth parameters in turkeys has not affected the quantitative antibody response, it has certainly reduced the potential of heavier birds to "fine-tune" their antibody response. The IgG antibodies are well known to be of much better affinity and avidity than are the IgM isotypes. The observation that modern turkeys are less able to switch their IgM-type antibodies to IgG-type antibodies may be one possible explanation for why heavier turkeys are more susceptible to pathogens, as described by Li et al. (2000a,b).

Lymphoproliferative Response to PHA-P

The modern 2003-strain turkeys did not differ or were only marginally better in toe-web thickness than the RBC2

Table 5. Lymphoblastic response against phytohemagglutinin phosphate¹ of modern commercial 2003 turkeys and 1966 randombred turkeys when fed representative 1966 and 2003 diets by strain, age, and diet.

Item	Diet ²	Sex	24-h increase (%)	48-h increase (%)
Strain ³				
2003	2003	Males	94.13	39.82
2003	1966	Males	102.48	63.89
1966	2003	Males	102.19	62.48
1966	1966	Males	78.39	54.88
2003	2003	Females	85.09	39.13
2003	1966	Females	111.31	78.54
1966	2003	Females	86.99	50.13
1966	1966	Females	78.81	49.70
Strain average				
2003			98.25	55.34
1966			86.60	54.30
Pooled SEM			8.62	7.00
Source of variation				
Strain			0.06	0.84
Diet			0.92	0.007
Sex			0.55	0.86
Strain × diet			0.009	0.0007
Strain × sex			0.56	0.12
Sex × diet			0.18	0.27
Strain × sex × diet			0.93	0.69

¹At 3 wk of age, phytohemagglutinin phosphate was injected at 100 µg/100 µL of saline per poult in the toe web of the right foot of 8 poults per strain-sex-diet subgroup. Swelling was measured by a constant tension micrometer at 0 (preinjection), 24, and 48 h postinjection. The increase in swelling was computed as the percentage increase in toe-web thickness from the preinjection value.

²2003 = turkey dietary regimen representative of those being fed in the 2003 calendar year; 1966 = turkey dietary regimen representative of those being fed in 1966, adapted from Ensminger (1967). Both starter diets were fed as crumbles.

³2003 = composite of 2 poults each of Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains; 1966 = randombred control strain (RBC2) from The Ohio State University (Wooster, OH).

birds when measured at 24 ($P = 0.07$) and 48 h post PHA-P injection (Table 5). Birds raised on the 1966 diets had a significantly higher toe-web swelling response when measured at 48 h post PHA-P injection ($P = 0.01$). Birds of the 2003 strain that were raised on the 1966 diets had a higher toe-web swelling response at 24 and 48 h post PHA-P injection ($P \leq 0.01$) than those raised on the 2003 diets.

A previous study showed T lymphocyte subpopulation differences between the F line and the RBC2 line (Li et al., 1999b, 2000c). The F line had a higher CD4 + CD8 T cell subpopulation than the RBC2 line. Past studies have shown a decreased toe-web response to PHA-P and a decreased in vitro mitogenic response to concanavalin-A in the F line compared with the RBC2 line (Bayyari et al., 1997; Li et al., 1999a). Although the PHA-P-induced toe-web swelling response is considered to be an indication of lymphoproliferative response in vivo, it is not clear which lymphocyte cell types may be involved in such a response. However, based on the studies by Li et al. (1999b, 2000c), one can argue that both CD4- and CD8-type lymphocytes may be involved. A differential susceptibility of these lymphocyte subtypes to mitogens may be the reason for variation or contrasting observations between the current study and the ones reported previously (Bayyari et al., 1997; Li et al., 1999a).

Mononuclear Phagocytic System Function Assessment

A mononuclear phagocytic system function assessment, in the form of colloidal carbon clearance from the blood

circulation, was determined at 12 and 13 d of age, and the data are summarized in Table 6. A comparative decline in the OD of carbon particles in the plasma indicates higher carbon clearance by the cells (i.e., blood monocytes and tissue macrophages) of the mononuclear phagocytic system. The modern strain of turkeys had significantly ($P = 0.01$) higher clearance of carbon particles at 5 min post colloidal carbon injection (PCCI) than did the 2003 strain. Males had a higher carbon clearance than did females ($P = 0.03$). A significant strain × sex interaction was present at 5 min PCCI ($P = 0.02$), where the difference between the 2003 males and females was greater than the difference between the males and females of the RBC2. A significant diet × sex interaction ($P = 0.01$) was also observed at 5 min PCCI, where the difference between the males on the 2 diets was in the opposite direction of the difference between the females on the 2 diets. These results are in contrast with a previous study in which phagocytic activity, as measured by the carbon clearance assay, was found to be lower in the high-growth-rate F line than in the RBC2 line (Li et al., 2001).

In summary, results of the present study indicate that the modern turkey, selected for higher BW, performs well in terms of bursa of Fabricius development. This is supported by the observation that no differences were observed in the total or IgM antibody response between the modern growth rate-selected 2003 commercial turkeys and the lower-BW RBC2 strain, which has been bred at random since 1966. However, our data suggest that the modern turkey may be at a disadvantage when it comes to switching IgM to IgG isotype antibodies. It is difficult to correlate

Table 6. Percentage increase in carbon particles in the blood circulation¹ of modern commercial 2003 turkeys and 1966 randombred turkeys when fed representative 1966 and 2003 diets by strain, age, and diet

Item	Diet ²	Sex	T5 ³ (%)	T20 ⁴ (%)
Strain ⁵				
2003	2003	Males	146.92	31.94
2003	1966	Males	209.15	36.10
1966	2003	Males	157.78	55.54
1966	1966	Males	208.26	35.58
2003	2003	Females	191.56	34.83
2003	1966	Females	163.10	35.28
1966	2003	Females	289.38	35.32
1966	1966	Females	236.77	37.14
Strain average				
2003			177.68	34.54
1966			223.05	40.89
Pooled SEM			23.80	5.61
Source of variation				
Strain			0.0118	0.13
Diet			0.66	0.42
Sex			0.027	0.33
Strain × diet			0.62	0.18
Strain × sex			0.025	0.22
Sex × diet			0.007	0.28
Strain × sex × diet			0.86	0.13

¹Colloidal carbon (india ink) was injected into the brachial vein of 6 poult/s/group at 100 µL/poult at 12 and 13 d of age. The level of carbon particles at 5- and 20-min intervals was measured in plasma samples via a microplate reader. The percentage increase in optical density (OD) of each sample, as an indicator of carbon levels over the baseline (T0) preinjection level, was calculated as follows: % OD = [(OD reading at given time – OD reading at preinjection)/OD reading at preinjection] × 100.

²2003 = turkey dietary regimen representative of those being fed in the 2003 calendar year; 1966 = turkey dietary regimen representative of those being fed in 1966, adapted from Ensminger (1967). Both starter diets were fed as crumbles.

³T5 = 5 min post colloidal carbon injection.

⁴T20 = 20 min post colloidal carbon injection.

⁵2003 = composite of 2 poult/s each of Nicholas Turkey, British United Turkeys of America, and Hybrid Turkeys strains; 1966 = randombred control strain (RBC2) from The Ohio State University (Wooster, OH).

this change with the actual resistance or susceptibility to a pathogen challenge because it was not performed in these studies. It was also encouraging to see that genetic selection for growth rate has not adversely affected the T cell-mediated response in modern turkeys. This observation implies that modern turkeys are equally competent at fighting off pathogen infections that require direct T lymphocyte engagement. Significant immune differences were seen on the basis of sex alone or as a result of an interaction with strain or diet. For example, the male birds were generally high responders for total and IgM-type antibody responses to SRBC during the booster phase of the antigen challenge (Tables 2 and 3). Furthermore, male turkeys demonstrated an increased clearance of colloidal carbon from the blood circulation at 5 min PCCI (Table 6). Hormones such as testosterone and estrogen are known to modulate immune responses, but the available literature is quite conflicting in terms of up- or down-regulation of both humoral or cell-mediated immune responses in a variety of species (Forsberg, 1984; Duffy et al., 2000). At the same time, sexual dimorphism in the immune response is well documented in the avian species (Norton and Wira, 1977; Marsh, 1992) as well as in rodents (Spitzer, 1999). Furthermore, genetic regions such as QTL have been identified that affect immune responses, such as antibody kinetics in female chickens (Zhou et al., 2003), as well as several other performance traits (Ankra-Badu and Aggrey, 2005).

Because the present study was conducted before the onset of sexual maturity, it would be insightful to investigate sex differences in the immune performance of sexually mature turkeys, as have been reported for other species (Cohn, 1979; Grossman, 1985, 1989; Gaillard and Spinedi, 1998; Møller et al., 1998). It would also be possible to localize the immune response differences to specific genetic regions once such information is available through the possible future genome sequencing or ongoing QTL discovery and identification studies in turkeys. The most encouraging implication of the data presented herein is an improvement in the function of the mononuclear phagocytic system in modern turkeys. This means that modern turkeys have maintained the nonspecific first line of defense mechanisms against pathogens, as indicated by a significantly better colloidal carbon clearance from circulation when compared with their RBC2 counterparts. In conclusion, it may be reasonable to suggest that the breeding companies continue to consider growth and immune response as coselection criteria in commercial breeding and selection practices.

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